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DAVID J. HINRICHS

January 1979

STUDIES ON THE ANTIGENIC COMPOSITION OF COXIELLA BURNETII

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The work described in this annual report is divided into three categories (1) definition of immune system in C. burnetii infection, (2) inference of mitogen character by use of LPS nonresponder mice and (3) initiation of effort to develop assays for the existence of cell-mediated immunity. The studies on immune mechanisms in C. burnetii all point to T cell control of the intracellular parasitism with an antibody dependent affect. The mitogenicity studies point to a lipoprotein as an important component in the antigen mosaic of C. burnetii.

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During the altered time period covered by this annual report we 1) have achieved a more complete definition of immunity in Q-fever, 2) have closed down our not so fruitful sub-unit vaccine studies on the mitogenicity and antigenicity of isolated C. burnetii components and 3) have changed our research orientation to more directly assess the role of cell-mediated immune mechanisms in C. burnetii and related intracellular parasite models in order to develop an appropriate in vitro test(s) which will measure the level of protective immunity that exists or develops in diseases controlled by cell mediated immune assays.

We have most recently started our efforts relative to item 3, above. The evaluation of cell-mediated immune mechanism and the development of appropriated in vitro tests has been initiated by some descriptive experiments conducted in the C_3H/HeJ mouse. This mouse strain which is a LPS nonresponder also does not reportedly respond to migration inhibition factor. We have carried out challenge experiments to determine if this strain of mouse differs in its intrinsic control of a classic cell-mediated immune disease. We found that this strain of mouse does differ in its initial capacity of developing immunity to Listeria monocytogenes. That is the C_3H/HeJ mouse could be killed by 10-50 fold fewer organisms than could other available strains of mice including Balb/c, C57Bl/6, outbred Swiss Webster and the LPS responder control strain C_3H/WSU derived from NIH. However, after immunity was established the C_3H/HeJ strain could handle as large a challenge dose of organisms as could any of the other strain following immunization. This may imply a defect in early cellular events in this strain or it may also imply that L. monocytogenes which also has mitogenic material as part of its cell wall structure affects the C_3H/HeJ strain differentially and this relates to the lowered LD_{50}.

We have prepared the lymphokines necessary to evaluate the potential of C_3H/HeJ to respond to MIF (nonresponder) and to macrophage activation factor (MAF). We have determined the level and activity of these lymphokines in Balb/c mouse macrophage assays and are now prepared to conduct these experiments in the C_3H/HeJ. The overall relationship of this effort to item 3) is outlined in the 1979 contract renewal proposal.

The C. burnetii mitogenic relationship has been evaluated in mice strains which now also include the C_3H/HeJ strain. As Table I indicated the LPS nonresponder does indeed respond to the mitogenic C. burnetii preparation in at least equal magnitude to the C_3H/WSU LPS responder strain. Additional confirmation of the B cell mitogen nature of these preparations can be seen by the induced response in lymphocyte cultures, derived from nude mouse lymphocyte cultures. Normal guinea pig lymphocytes do not respond to the C. burnetii preparations which is a result seen for most of the standard LPS, bacterial derived, preparations.

It is apparent that the standard preparations of C. burnetii have a substantial mitogenic effect. Evidently the TCA extractable material is more enriched in the mitogen component relative to the intact organism preparations. The nature of this mitogen due to its effect on the C_3H/HeJ mouse, is not Lipid A of the Enterobacteriaceae relationship. By analogy the C. burnetii mitogen may be more lipoprotein in nature since the C_3H/HeJ will respond to phenol extracts of E. coli LPS. Our early work on antigen definition of TCA soluble material indicated an antigenic protein component in these extracts. It is of course well established that the TCA extractable material is also rich in lipid and consequently a mitogenic lipoprotein is a plausible explanation for the strain independent mitogenicity of these preparations. Although we have been asked to

TABLE I

The Response of C_3H/HeJ and C_3H/WSU* Mice Strains to Preparations of C. burnetii

Mitogen	Mouse Strain C_3H/HeJ S.R.**	C_3H/WSU S.R.	nu/nu
Con A 0.2 g	52 8	38 14	0.8
WCP-I 5 g	3.6	4.2	3.3
WCP-I 25 g	5.7	6.5	4.8
WCP-II 5 g	9.2	6.8	6.6
WCP-II 25 g	11.1	9.2	9.0
TCA-I 1 g	9.4	9.6	4.1
TCA-I 5 g	2.2	1.8	3.7

*C_3H/WSU is a locally maintained strain originally obtained from NIH to Dr. K. L. McIvor.

**S.R. = stimulation ratio calculated ratio cpm stimulated/cpm control. Lymphocyte cultures were spleen derived, cultured in round bottomed microtiter plates,
Cell density 2 x 10^5/culture, 0.2 ml/culture, cultured at 37°C, 5% CO_2-95% air in RPMI-1640 2% fetal calf serum. ^{3}H-thymidine label last 24 hrs of culture.
WCP-I -- intact C. burnetii harvested from embryonated eggs, these are Phase I organisms.
WCP-II -- intact C. burnetii harvested from embryonated eggs, these are Phase II organisms.
TAC-I -- trichloroacetic acid extractable material from Phase I organisms, material was extensively dialyzed and then lyophilized prior to use.

channel our efforts away from a definition of the complex antigenic mosaic of C. burnetii it does seem that lipoprotein material is a likely candidate as the major immunogenic component of this rickettsia. Difficulties in obtaining large amounts of this material will discourage the casual researcher in investigation of the influence of this macromolecule, however this may prove to be extremely important information relative to effective vaccine consideration in general and in C. burnetii vaccines specifically.

We have just completed a multiphased study which has been designed to define the role of cellular immunity in C. burnetii infections in the mouse and by extension to other animal species. Our total effort in this regard, the complete results of which are now being collected, has examined the clearance of vialbe phase I C. burnetii from infected mice. We have employed in these studies spleen (and in many cases liver) impression smears to assay for infection, and in earlier phases of the study verified the viability of the spleen contained rickettsia by egg infectivity. In these studies we wanted to establish what role T lymphocytes had on immunity to C. burnetii in the mouse and to compare the role of these cells to the effects of antibody to C. burnetii in the same system.

In order to establish the effect of various combinations of immune potential we set up experiments to 1) examine the effect of passively transferred anti-C. burnetii antibody on recipient clearance of C. burnetii, 2) examine the effect of passively transferred spleen cells, obtained from normal and immune mice, on recipient clearance of C. burnetii, 3) examine the clearance rate and antibody levels in athymic (nu/nu) C. burnetii infected mice and 4) examine the clearance rate of C. burnetii in athymic following reconstitution with nu/+ normal thymus cells. In general, and as indicated in Table II, every observation in the four experimental groups is consistent with a cellular mechanism responsible for immunity.

It is evident from this table that successful C. burnetii clearance follows classic cell-mediated immune expectations. The inability of the nude mouse to clear C. burnetii in the presence of actively produced high levels of antibody, coupled with the observation that passive transfer of serum into normal mice did not affect the kinetics of C. burnetii clearance indicated the role of antibody in this infection. Just as obvious is the essential role that lymphocytes (undoubtedly T lymphocytes) play in C. burnetii immunity. In normal mice that have a complete immune system rickettsial clearance is usually complete in 21 days. WCP-I vaccinated animals or animals following viable infection rapidly clear challenge doses of C.burnetii. Normal animals that have been the recipients of 10^8 immune spleen cells prior to challenge will also clear the challenge dose in an accelerated fashion. Finally the nu/nu mouse, which lacks functioning T cells does not control the intracellular parasitism of C. burnetii even though this athymic mouse can produce high levels of antibody. This antibody is undoubtedly produced because of the associated B-cell mitogenicity of C. burnetii (mentioned above) which would provide the needed stimulus for full cycle antibody production. We feel that the reconstituted nude mouse, which can also very effectively clear the challenge dose of C. burnetii, offers additional support for the cell-mediated nature of the immune mechanism that controls and eliminates C. burnetii. There are still some loose pieces to be picked up in these series of experiments and I would think some disclaimers might be heard, however the consistency of the observations to date certainly imply a very obvious role for T-cell function in C. burnetii immunity.

TABLE II

The Role of Passively Transferred Cells and Antibody in C. burnetii Clearance

Animal Group 3-7 mice/group	Days Post Infection 7	14	21
[1]Normal*	3+	3-4+*	0-1+
[2]Immune-A	2+	0-1+	0
[3]Immune-B	2+	0-1+	0
[4]Normal AB recip	2+	3+	1+
[5]Normal NS recip	3+	4+	2+
[6]Normal IC recip	1+	1+	0
[7]Normal NC recip	3+	3+	1+
[8]nu/nu	3+	4+	4+
[9]nu/nu TC recip	2+	1+	not determined

*Spleen impression smears were stained with Gimenez stain and rickettsia were determined according to the following evaluation.

0 - no rickettsia present.
1+ - occasional cells with rickettsia, not apparent in every field.
2+ - many cells with rickettsia, some with greater than 50 rickettsia/cell infected cells apparent in most fields.
3+ - all cells in every field with obvious rickettsia.
4+ - as for 3+ with free rickettsia throughout the impression.

2 - mice 3 weeks following recovery from C. burnetii infection.
3 - mice 14 days following single I.P. injection of 100 g WCP-I.
4 - mice injected I.P. with 0.5 ml of anti-WCP-I antibody that had a microagglutination titer of 1024.
5 - mice injected I.P. with 0.5 ml normal mouse serum.
6 - mice injected I.V. with 10^8 spleen cells obtained from mice that have recovered from a C. burnetii infection (5 weeks post infection).
7 - mice injected I.V. with 10^8 spleen cells obtained from normal mice.
8 - athymic mice (at 21 days these mice had an average anti-WCP-I antibody titer of 2048).
9 - athymic mice reconstituted with 10^8 thymus cells obtained from nu/+ mice reconstitution 24 hrs prior to C. burnetii challenge.

The early date requested for contract renewal efforts (which also requires this annual report) precludes the addition of completed manuscripts that should accompany this report. These manuscripts, which will form the basis of the doctoral dissertation of one of my graduate students are in only the early stages of preparation. We hope to submit these completed manuscripts in April for publication in the *Journal of Infection and Immunity*. We feel that these completed studies will point to the potential control that cell-mediated immunity expresses in the host-parasite relationship that involves obligate and intracellular parasites. This should become more obvious in other rickettsial systems.

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